Effect of coal mine pit water on the productivity of cattle II.* Effect of increasing concentrations of pit water on feed intake and health

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Abstract. The effect of drinking high mineral content coal mine pit water on the health and growth of yearling tropically adapted steers was investigated. Steers consumed town water ($\sim$30 mg sulfate/L) or dilutions of pit water, which at the highest concentration contained (mg/L) 4000 sulfate as well as 3082 chloride, 328 calcium, 562 magnesium, 2600 sodium, and other minerals at lesser concentrations (total dissolved solids, 8600 mg/L). The growth and performance of the steers were measured as average daily weight gain, dry matter intake, faecal dry matter content, and water intake. Health was assessed using haematological indices (packed cell volume, haemoglobin, and others) and on randomly selected animals, by complete *post mortem* haematological and biochemical analysis.

Consumption of diluted pit water of up to 2000 mg sulfate/L, if introduced gradually, did not result in a reduction in dry matter or water intake. Significant interactions ($P < 0.05$) occurred between rate of introduction and plane of nutrition in affecting weight gain, whereby weight gains on pit water were marginally greater when treatment was introduced abruptly. Plane of nutrition was the main effect in determination of packed cell volumes, where low plane of nutrition led to higher values. Interactions of time on pit water treatment with rate of introduction or nutrition in affecting packed cell volume were statistically significant ($P \leq 0.006$) but small in magnitude (1–2%), and hence unlikely to be biologically significant since averages remained within the normal range for the age group. Pit water treatment did not compromise the animals' health at 2000 mg sulfate/L, as assessed by visual veterinary and histopathological examinations of tissues taken at autopsy. When the concentration of pit water was increased to 4000 mg sulfate/L, dry matter intake was depressed by 14% and water intake was decreased by up to 40%, increasing slightly with longer time on treatment. Under the conditions of this experiment, beef steers can drink coal mine pit water containing up to 2000 mg sulfate/L (4000–6000 mg/L of total dissolved solids) without suffering ill effects, provided that it is introduced gradually. The study therefore provides evidence that the recommendation of 1000 mg sulfate/L as the maximum concentration in livestock drinking water may be too conservative for steers if favourable conditions exist.

Additional keywords: drinking water, sulfate, growth, health.

Introduction

In a number of locations in Australia, open-cut mining and beef production coexist. Water that accumulates in coal mine pits usually has a high mineral content as a result of salts leaching from surrounding parent material (BHP 1991). Cattle can drink this water, especially during drought, but the potential for adverse health effects obviously exists. The current guidelines for tolerances of cattle to mineral contami-

nation in drinking water are based on field observations and not on experimentation (ANZECC 1992).

The geology of the Bowen Basin in Central Queensland is such that coal seams are covered by sedimentary rock (BHP 1991), a structure that predisposes the ground water and rainfall runoff from coal mines to varying, but predominantly high, levels of sulfate. Once dissolved in rainwater, sulfate is a stable solute unless acted upon by anaerobic microorganisms (Veenhuizen and Shurson 1992). Hence, sulfate ion in particular would be of interest in developing guidelines for use of Central Queensland coal mine pit water in animal production. The other metal ions present may also be of concern (chloride, magnesium, calcium, sodium, cadmium, arsenic, and lead).

The tolerances and toxicities of sulfate in livestock have previously been studied (Pierce 1960; Weeth and Hunter 1971; Weeth and Capps 1972; Digesti and Weeth 1976; National Research Council 1980; Kandylis 1984; Veenhuizen and Shurson 1992; Robertson *et al.* 1996). Weeth and Hunter (1971) found that heifers drinking water containing 3493 mg sulfate/L had a 30% lower feed intake than controls. Later work by Weeth and Capps (1972) reported that 2814 mg sulfate/L in drinking water depressed feed intake. Growth rate was at most transiently affected. There was a need for larger scale studies to investigate the possibility of interactions between rate of introduction, nutrition, or pre-exposure, and diluted pit water treatments, in terms of standard animal production indices.

This paper reports experiments into the effects of diluted coal mine pit water on steers. Expts 1–4 were designed to test the hypothesis that animal health and growth are influenced by (*i*) the concentration of the pit water, (*ii*) the rate of introduction (abrupt *v.* gradual), (*iii*) the plane of nutrition during the exposure, or (*iv*) some interaction between these conditions. Of particular interest were the long-term effects of high mineral intake on the functioning of vital organs and the accumulation of minerals in edible tissues. Expt 1 was extended (1*a*) to test the effects of higher concentrations of pit water on the health and growth of steers that had already been exposed to lower concentrations of pit water.

Materials and methods

Experimental animals

In total, 88 healthy yearling steers were purchased and recruited into 5 experiments over 18 months. The design of the experiments is shown in Table 1, along with details of the experimental groups. The steers used were Brahman, except in Expts 1 and 1*a*, which used Belmont Red steers. The animals in Expts 1–4 had no prior exposure to coal mine pit water. The steers were housed in a roofed animal house at the Tropical Beef Centre, Rockhampton, for the duration of the experiments. Steers were free of the cattle tick (*Boophilus microplus*) and were treated to control gastrointestinal helminths prior to the commencement of the pit water treatments.

Pit water

Coal mine pit water was transported from a Central Queensland mine to the Tropical Beef Centre and stored in bulk in fibreglass tanks. The practicalities of handling large volumes of water dictated the use of 2 batches of coal mine pit water. The compositions of these are defined in Table 2. Daily dilutions of the coal mine pit water stock were made with town water to specified concentrations of sulfate, which was chosen as the reference solute. When required, pit water was transferred to a covered in-ground concrete tank and mixed by bubbling compressed air through it for 1 h. The term 'coal mine pit water' is used to describe the undiluted fluid obtained from the mine, and 'pit water' is used to describe the fluid diluted with town water, for use in experiments.

Experimental procedures

The animals were weighed and randomly allocated to groups so that the group mean weights were approximately the same. These groups were then randomly allocated to treatment. The group mean weights (±s.e.m.) at the start of each experiment are shown in Table 1. At the end of Expt 1, 3 animals from each group were chosen at random, following standard procedures. They were slaughtered, and detailed *post mortem* examinations were performed. The other 15 steers (one from the control group was removed from the trial due to lameness) remained in the treatment groups established for Expt 1 and consumed the same concentrations of pit water. After 3 weeks, the treatment concentrations were increased as described below.

Table 1. Summary of experiments

Expt no.	Plane of nutrition	Rate of exposure	Treatment period at final concentration (weeks)	Pit water concentrations (mg/L)	Animals per group	Starting weights (kg)	Pit water batch
1	High	Gradual	15[A]	30, 500, 1000, 2000	7[B]	254±4	1
2	High	Abrupt	6[C]	30, 500, 1000, 2000	5	269±4	2
3	Low	Gradual	9[D]	30, 500, 1000, 2000	5	349±7	2
4	Low	Abrupt	6[E]	30, 500, 1000, 2000	5	277±5	2
1*a*	Low	Gradual	8[F]	30[G], 2000, 3000, 4000	4[B]	357±3·5	1

[A] Started 6 April 1993. [B] Groups housed in group pens which meant that dry matter intake and water intakes were group average measurements. [C] Started 9 January 1995. [D] Started 6 July 1994. [E] Started 21 September 1994. [F] Started 7 July 1993. [G] This group had only 3 animals.

Table 2. Town and coal mine pit water chemical composition

Measurement	Town water[A]	Coal mine pit water Batch 1	Batch 2
Electrical conductivity (mS/cm)	350	18 200	16 000
Total dissolved solids (mg/L)	200	11 935	10 326
pH	8·2	8·1	8·3
Cl^- (mg/L)	50	4300	3800
$SO_4{}^{2-}$ (mg/L)	30	5580	3428
Hardness as $CaCO_3$ (mg/L)	100	43 700	1925
Other minerals (mg/L)			
Ca	23	460	194
Mg	11	784	350
Fe	<0·02	<0·02	<0·05
Mn	<0·02	<0·01	0·1
Na	30	n.m.	2180
K	4	n.m.	19
Cu	<0·1	0·4	<0·1
Zn	<0·2	0·8	<0·05
Pb	<0·1	<0·10	<0·1
Al	<0·1	n.d.	<0·3
Cd	n.m.	6·8	<10
As	n.m.	<2	<2

n.m., not measured.
n.d., not detected at instrument detection limit.
[A] Average values calculated from data measured 2-monthly over a 2-year period encompassing this experiment series. Measurements were made by the Rockhampton City Council.

Pit water treatments

The coal mine pit water was diluted with town water, such that the final concentrations (defined for convenience in terms of sulfate concentration) were 30–4000 mg/L (Table 1). In Expt 1a, animals previously exposed to 500 mg sulfate/L in Expt 1 became the treatment group exposed to 2000 mg sulfate/L. The previous 1000 and 2000 mg/L groups were stepped up to 3000 and 4000 mg/L, respectively. The duration of treatment is indicated in Table 1.

Nutritional status

There were 2 nutritional planes, high and low (Table 1). The high nutritional plane was achieved by feeding steers *ad libitum* with air-dried, long-chopped lucerne (*Medicago sativa*) hay. The amount of feed offered was such that the daily residue was 0·8–1·5 kg. The low nutritional plane was achieved with *ad libitum* long-chopped Angleton grass (*Dicanthium aristatum*) hay. A supplement of 500 g linseed meal/steer·day was included in all rations of Expt 1a animals.

Rate of introduction

The pit water was introduced to the animals either gradually or abruptly (Table 1). In the gradual introduction experiments, the water started at an initial concentration of 500 mg/L, and was increased by 500 mg/L every 7 days until final concentrations were achieved, after which time that concentration was maintained. Steers then had access to water of the required concentration for the remainder of the experiment. The times required to reach the final concentrations of 1000 and 2000 mg sulfate/L were therefore 14 and 28 days, respectively. Animals in Expt 1a were introduced to higher concentrations of pit water at an equivalent rate of 500 mg/L every 7 days.

Animals in the abrupt introduction experiments were offered pit water at the defined concentration from the first day of the treatment period.

Animal measurements

Steers were weighed twice weekly before feeding. Average daily liveweight gain (ADWG) was measured by linear regression analysis over 4 successive measurements. Samples for laboratory analysis were collected according to procedures outlined in the Queensland Department of Primary Industries Veterinary Laboratories User Guide. Samples of venous blood were obtained by jugular venipuncture at 7-day intervals using vacutainers containing sodium heparin. Packed cell volume (PCV) was measured using a capillary of blood, and larger volumes of blood were centrifuged for separation of plasma. Two aliquots of plasma were stored, one at $-80°C$ for biochemical analysis and the other at $-20°C$ for sulfate determination. Faeces for dry matter determination was obtained from individual steers by rectal grab sampling at 7-day intervals. Feed intakes for Expts 1–4 were measured by weighing feed into the animals' stalls and recording any residues. Water intakes for Expts 1–4 were measured using flow meters attached to individual drinkers in the stalls. The latter 2 indices were measured for the groups of animals in Expt 1a.

Biochemical and haematological analyses

Histopathology and blood biochemistry analyses were performed using standard procedures as defined in the Queensland Department of Primary Industries Veterinary Laboratory Procedures Manual. Blood smears were made from the EDTA blood for haematological determinations.

Histopathological examinations were undertaken on the following tissues: parotid salivary gland, trachea, lung, heart, liver, spleen, kidney, adrenal glands, pancreas, rumen, reticulum, omasum, fundic abomasum, pyloric abomasum, duodenum, jejunum, ileum, caecum, colon, urinary bladder, skeletal muscle, and skin.

The following biochemical indices of blood were measured on samples from Expt 1: calcium, total protein, globulin, bilirubin, urea, creatine phosphokinase, glutamate dehydrogenase,

magnesium, albumin, albumin to globulin ratio, creatinine, gamma glutamyltransferase, and aspartate aminotransferase.

The following haematological indices were measured on samples from Expt 1: haemoglobin, packed cell volume (PCV), erythrocyte density, mean erythrocyte volume, mean erythrocyte haemoglobin, mean corpuscular haemoglobin concentration, leucocyte density, segmented neutrophil density, lymphocyte density, monocyte density, and eosinophil density.

Samples of liver, kidney, muscle, and adipose tissue were collected at *post mortem* examination, for inorganic analysis. Samples for total sulfur analysis were digested with nitric–perchloric acid prior to measurement by autoanalyser using the technique of Mottershead (1971) and sodium sulfate as standard. Na, Ca, Mg, Cd, Pb, and As were determined by atomic absorption spectrophotometry (AAS; Varian Spectra A-300) on nitric acid digested samples. Na, Ca, and Mg concentrations were determined using flame emission. Vapour generation AAS was required for As and a graphite furnace AAS for Cd and Pb. A National Bureau of Standards Bovine Liver standard with certified values for each of these elements was run concurrently with the experimental samples. Na, Ca, and Mg values were within 7% of the quoted standard. Cd and Pb values were within 50% of the quoted standard, and As was below the detection limits of the AAS. In the experimental samples, As was detected as AsH_3. Coal mine pit water concentrations of Fe, Mn, Cu, Zn, and Al were also determined by AAS. Sulfate and chloride concentrations were detected using turbidometric and coulimetric techniques respectively.

Dry matter contents of feed and faeces were determined by drying at $100°C$ to a constant weight in a forced draught oven.

Statistical analyses

The ADWG, faecal dry matter content, and PCV data from Expts 1–4 were analysed together by repeated measures analysis of covariance using a factorial design. The dataset included these indices for each animal, for the 6 weeks following achievement of the final treatment concentration of pit water. This analysis allowed investigation of effects of time on treatment, as well as interactions between experimental variables. A protected least significant difference test was applied to the adjusted means. Animal liveweight and PCV at the start of each experiment were included as potential covariates. These data were analysed using the general linear models procedure in SAS (1988). Dry matter intake and water intake could not be analysed this way because Expt 1 was performed in group pens. These indices were analysed for each experiment individually, by analysis of variance as a split plot in time (Snedecor and Cochran 1989), using the GENSTAT (1988) program. The data set consisted again of the animal group indices measured for the 6 weeks following achievement of final treatment concentrations of pit water. For Expt 1*a*, all animal indices from each experiment were analysed using the above-mentioned GENSTAT procedure.

Results

Given the variation between samples from different coal mine pits at different times (Table 2), one solute (sulfate) was chosen as the reference upon which dilutions were standardised. Sulfate has therefore become the focus of experimentation; however, it was recognised from the outset that other constituents may be the source of any effects. The effects of other

Table 3. Effect of pit water treatment, time on treatment, plane of nutrition, and rate of introduction on the average daily liveweight gain of steers

Adjusted means (adjusted to equalise variance between time points) and s.e.m. (in parentheses) presented; within columns, means followed by the same letter are not significantly different at $P = 0.05$

	Time on treatment (days)						Overall
	7	14	21	28	35	42	
Expt 1	0·9a	1·1a	1·1a	1·0a	1·2a	0·8a	
	(0·1)	(0·1)	(0·1)	(0·1)	(0·1)	(0·1)	
Expt 2	1·5c	0·7c	2·0c	0·7a	1·2c	1·4c	
	(0·1)	(0·1)	(0·1)	(0·1)	(0·1)	(0·1)	
Expt 3	−0·6b	−0·7b	0·7b	−0·4b	−1·0b	−0·6b	
	(0·1)	(0·1)	(0·1)	(0·1)	(0·1)	(0·1)	
Expt 4	0·5b	0·3d	−0·5b	−0·2b	−0·9b	0·3d	
	(0·1)	(0·1)	(0·1)	(0·1)	(0·1)	(0·1)	
Main effects							
Rate of introduction (RI)	***	n.s.	***	n.s.	n.s.	***	***
Plane of nutrition (PN)	***	***	***	***	***	***	***
Time	—	—	—	—	—	—	***
Interactions							
RI×PN	***	***	***	n.s.	n.s.	***	*
RI×time	—	—	—	—	—	—	***
PN×time	—	—	—	—	—	—	***
Potential covariates							
Liveweight at Time 0	—	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

$*P < 0.05$; $***P < 0.001$; n.s., not significant.

constituents of coal mine pit water will be addressed more specifically in another study.

Experiments 1–4

There was no significant association between pit water concentration and ADWG.

Rate of introduction to pit water interacted with plane of nutrition in affecting ADWG, in that animals abruptly exposed to pit water had significantly greater ADWG than those gradually exposed to pit-water ($P < 0.001$). This significant interaction was observed at Days 7–21 ($P = 0.02$), as well as over the 6-week period ($P = 0.02$). Animals on a high plane of nutrition with abrupt introduction to pit water, independent of pit water concentration, yielded the greatest ADWG ($P = 0.002$). The fact that the animals in Expt 3 were heavier at the start of the experiment could have affected the measured ADWG, although animal liveweight at the start of the study was not a significant covariate.

Time on treatment also had a significant effect overall and, more importantly, interacted with plane of nutrition as well as rate of introduction to influence ADWG. ADWG varied widely with time in the groups on a low plane of nutrition, whereas the growth of animals on the high plane of nutrition was more consistent (Table 3). ADWG also varied widely and significantly when treatment was introduced abruptly as opposed to gradually.

PCV measurements were used as simple indicators of the haematological status of the animals during the course of each experiment. Pit water treatment *per se* did not have a significant effect on PCV. Nutrition had the main effect on PCV, where the adjusted average PCV of steers on the low plane of nutrition was greater than that of steers on the high plane for the entire 6 weeks (46 ± 1.2 *v.* 35 ± 0.9, respectively; $P < 0.001$). Animal liveweight early in the study was a significant positive covariate in this analysis at all times (excluding Day 28) and overall ($P = 0.01$). Interactions of time on treatment and rate of introduction or nutrition in association with PCV were statistically significant ($P \leq 0.006$), but small in magnitude (1–2% PCV), and were unlikely to be biologically significant as no consistent pattern was apparent within the variable combinations. Rate of introduction had a significant effect on Day 28 only, when animals that were abruptly exposed to pit water had significantly greater PCV than those gradually exposed to pit water (1% PCV; $P = 0.006$).

Increasing the concentration in pit water by gradual introduction did not significantly alter 3 other indices of animal nutrition: dry matter intake, water intake, faecal dry matter content (Table 4). In contrast, abrupt introduction to increased concentrations of pit water, at a high plane of nutrition, was associated with significantly decreased dry matter intake, but only at the highest concentrations (Expt 2, $P < 0.05$). Furthermore, abrupt introduction at a low plane of nutrition was associated with increasing water intake (Expt 4, $P < 0.05$). Faecal dry matter contents from all animals in Expts 1–4 were also analysed as functions of pit water treatment, time on treatment, rate of introduction, and plane of nutrition. In this case there were no significant effects of experimental variables on the variation in faecal dry matter contents ($P > 0.1$, not shown). Initial liveweight of the animals was, however, a significant ($P < 0.05$) covariate to faecal dry matter content at all times during the experiment, except Week 5.

Table 4. Dry matter intake, water intake, and faecal dry matter of steers over six weeks of pit water treatment

Expt no.	Sulfate (mg/L)				s.e.m.	Significance
	30	500	1000	2000		
Dry matter intake (g/kg LW·day)						
1	29·1	28·5	29·8	28·5	0·9	n.a.
2	27·4	26·0	26·0	23·3	0·3	$P < 0.05$
3	9·6	9·8	10·4	10·0	0·4	n.s.
4	11·0	11·5	12·7	11·5	0·1	n.s.
Water intake (mL/kg LW·day)						
1	127	123	130	130	4	n.a.
2	135	121	138	138	2	n.s.
3	31	45	38	34	5	n.s.
4	35	35	55	47	2	$P < 0.05$
Faecal dry matter content (%)						
1	13	14	12	13	0·6	n.s.
2	15	15	14	15	0·4	n.s.
3	22	22	23	22	1·0	n.s.
4	24	24	22	21	1·0	n.s.

n.a., not assessed statistically, owing to the use of group pens.
n.s., not significant at $P = 0.05$.

Table 5. Mineral concentration of fresh tissues from three steers of control and 2000 mg sulfate/L treatment groups in Experiment 1

Means and s.e.m. (in parentheses) presented

Tissue	Treatment group	S (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Cd ($10^2 \times$ mg/kg)	As (μg/kg)	Pb (μg/kg)
Muscle	Control	1160 (50)	380 (30)	35 (1)	56 (1)	4 (0·5)	1 (1)	90 (20)
	Sulfate	1440 (5)	390 (2)	35 (1)	57 (3)	4 (0·2)	1 (1)	120 (20)
Fat	Control	n.m.	280 (25)	13 (1)	8 (1)	2 (2)	2 (2)	15 (8)
	Sulfate	n.m.	340 (60)	17 (4)	10 (2)	1 (0·2)	2 (2)	20 (13)
Liver	Control	1900 (90)	630 (30)	36 (1)	170 (9)	1 (0·1)	3 (2)	210 (45)
	Sulfate	1910 (20)	620 (70)	37 (2)	185 (8)	1 (0·2)	1 (1)	210 (38)
Kidney	Control	1500 (90)	1640 (80)	78 (6)	165 (4)	3 (0·6)	3 (1)	120 (26)
	Sulfate	1534 (5)	1870 (60)	100 (12)	165 (4)	4 (0·8)	10 (10)	125 (27)
Maximum residue limits (NHMRC 1987)								
Muscle						20	1000	1500
Fat							1000	1500
Liver						125	1000	1500
Kidney						250	1000	1500

n.m., not measured.

Water and dry matter intakes of control cattle (30 mg sulfate/L) were decreased on the low plane of nutrition relative to the high plane (Table 4, Expts 3 and 4 *v.* 1 and 2). Further, water intakes of control animals were marginally higher in Expt 2 than Expt 1, which may have resulted from higher ambient temperatures. Water intakes were within the expected ranges based on the dry matter intakes and the recommendations of the Standing Committee on Agriculture (SCA 1990) and NRC (1981).

Post mortem examinations of 3 steers from each treatment group of Expt 1 revealed no gross pathological changes in any of the animals. There were no significant differences between treated and control animals for most of the haematological and biochemical indices (Appendices 1 and 2). Two animals from the 500 mg/L group and 2 from the 2000 mg/L group exhibited elevated creatine phosphokinase levels: 1815, 3690, 1224, 921 IU/L; normal range 10–200 IU/L. Whilst this suggests muscle breakdown, the randomness of occurrence and the lack of a consistent treatment effect lead us to suggest that the animals may have been stressed immediately prior to slaughter.

Treatment differences in the concentration of each mineral in the tissues examined were not significant (Table 5, detailed analysis not shown).

Experiment 1a

In this experiment, animals that had already been exposed to pit water were kept in the same treatment groups, but gradually exposed to higher concentrations of pit water. Since dry matter intake and water intake of the animals were measured on the animals as groups, the statistical analysis of these indices is more limited than in Expts 1–4. However, since time on treatment

had been a main effect in Expts 1–4, we reasoned that time may again be a valuable indicator of physiological effects of treatment.

No animals showed any signs of ill health or digestive upset, such as diarrhoea, during the course of the experiment. Dry matter intake was depressed in steers consuming pit water when concentrations were increased to $\geq$2000 mg sulfate/L. At 4000 mg/L the depression was 14% averaged over time (Table 6), and up to 30% at 1·5 weeks (Fig. 1), although after this point there was little time dependence. Water intake of the same groups was reduced by treatment (up to 40% at 4000 mg/L) when averaged over the experiment, but slowly increased over time. Group differences that existed at the start of the experiment persisted throughout, suggesting an effect of treatment history.

Faecal dry matter content showed a trend towards higher values with increasing pit water concentration (Table 6, Fig. 1) but this was not significant ($P = 0·055$). The faecal dry matter content in the week prior to the treatment reaching final concentration (defined as zero time) was a significant covariate for the faecal dry matter percentages at Day 21, 35, and 42 ($P = 0·02$). Hence, variation seen in the groups during the experiment reflected variation that existed in the experimental groups prior to establishment of treatment. The downward trend in faecal dry matter content was reflected generally amongst the treatment groups. At the highest treatment concentration there was a downward trend in the values with time on treatment (Fig. 1).

Packed cell volume showed no significant treatment effect ($P = 0·067$), although as with faecal dry matter content, the zero time value was a significant covariate ($P = 0·001$) for all subsequent measurements. There
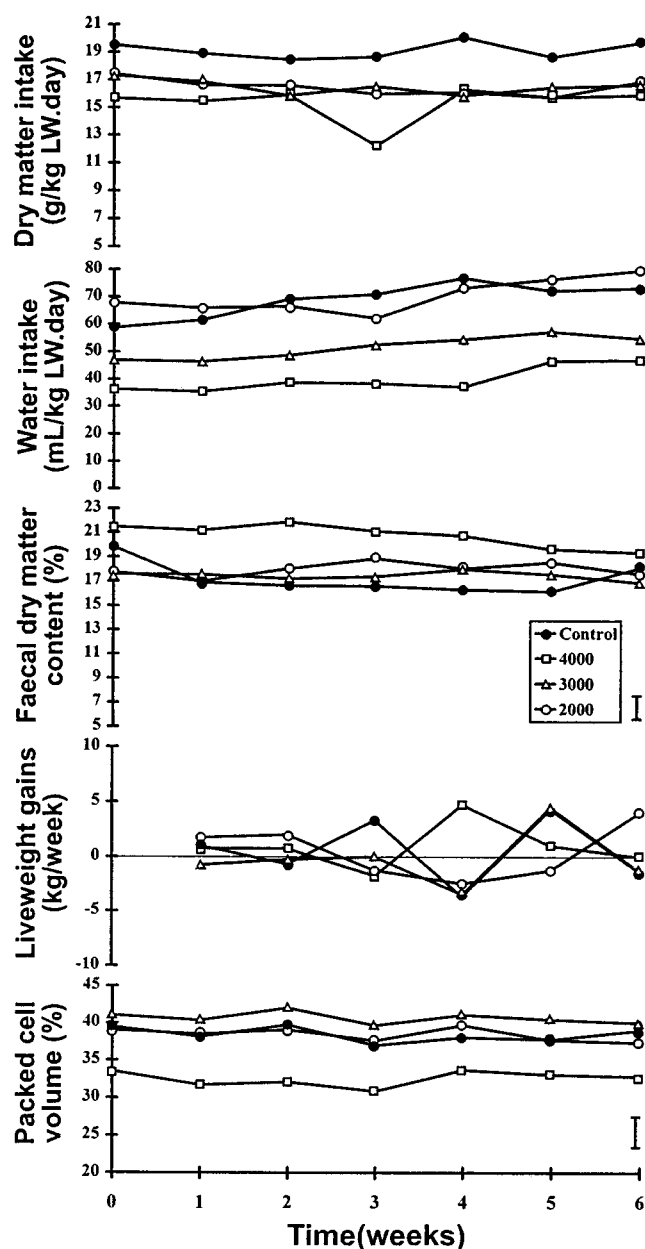
Fig. 1. Indicators of animal nutrition and physiology for 4 animal groups as a function of time on coal mine pit water. The five diagrams within the figure represent the control ● (town water) and treatments of 2000 mg/L (○), 3000 mg/L (△), and 4000 mg/L (□) of sulfate, final concentration. Times represent weeks after attainment of the final treatment, sulfate concentration. The indicators are from the top plate: dry matter intake, water intake, faecal dry matter content, average ADWG and packed cell volume.

was a significant ($P = 0.008$) association between pit water concentration and time on treatment, where PCV decreased during the early weeks of the highest concentration treatment (Fig. 1).

As the result of feeding steers a diet that approximated maintenance, ADWG was lower than in the previous experiments (Table 6, Fig. 1) and very variable over time. Animal liveweight at the start of the study was a significant positive covariate for ADWG on treatment ($P = 0.05$). There was no significant association between pit water concentration and ADWG and no significant or apparent trend with time on treatment.

Discussion

Diluted coal mine pit water was not toxic* to cattle when concentrations of sulfate in these experiments were below 2000 mg/L (4000 mg/L of total dissolved solids). Even at concentrations of 2000–4000 mg sulfate/L, the steers did not demonstrate overt signs of toxicity. Removal of animals from the experiment, which occurred in only one case, was on the basis of health effects unrelated to treatment, i.e. one animal was removed from the 30 mg/L group of Expt 1 for lameness. There were significant changes in animal physiology as indicated by the production indices, but these coupled with a battery of biochemical and histopathological analyses did not suggest toxicosis or poisoning even after 3 months with diluted pit water as the sole source of drinking water.

Table 6. Expt 1*a*. Dry matter intake, water intake, faecal dry matter, packed cell volume, and ADWG of steers

| | Sulfate (mg/L) | | | | s.e.m. | Sign. |
	30	2000	3000	4000		
Dry matter intake (g/kg LW·day)	18	16	16	16	0·6[A]	n.a.
Water intake (mL/kg LW·day)	71	71	52	40	7[A]	n.a.
Faecal dry matter content (%)	17	18	17	21	1	n.s.
Packed cell volume (%)	38	38	41	32	2	n.s.
ADWG (kg/day)	0·4	−0·1	−0·2	0·3	0·1	n.s.

n.a., not assessed statistically; animals studied as a group.
n.s., not significant at $P = 0.05$.
[A]Calculated on the groups, across the time period.

The experiment reflects typical diets and some of the conditions that grazing animals in central Queensland experience and, where potentially exposed to coal mine pit water, would be likely to encounter. The range of concentrations of the pit water was based on both predictions of the concentrations encountered by cattle in the field, and the known levels above which toxic effects might be encountered (Pierce 1960; Weeth and Hunter 1971; Weeth and Capps 1972; Digesti and Weeth 1976; National Research Council 1980; Kandylis 1984; Veenhuizen and Shurson 1992). The

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* The definition of toxic presented in Butterworths Medical Dictionary is related to poison, which is 'a noxious substance which, by its action on organs or tissues of the body, can impair function or destroy life'.

diets represent the extremes of nutritive value offered in the region. Whilst animals experienced the same ambient temperatures as in the field, the provision of covered pens would no doubt affect their ability to cope with these conditions.

Of interest in the context of animal production was the accumulation of minerals in edible tissues, as this may have affected meat quality or food safety. Although the pit water contained traces of lead, cadmium, and arsenic, these did not accumulate in edible tissues at levels above the maximum residue limit. Even though cadmium was difficult to determine accurately, a large margin exists between the measured values and the maximum residue limit. Similarly, despite water-borne intakes of sulfate, chloride, sodium, magnesium, and calcium up to 70 times that of control steers, there was no accumulation in edible tissues, which suggests that at least in terms of the minerals analysed, food safety was not compromised.

In terms of the physiology of cattle, this study has identified some interesting responses. It was clear that under animal-house conditions, cattle were able to tolerate quite high mineral content in water over extended periods without adverse effects. Pit water exerted its physiological effects under specific circumstances of nutrition, rate of introduction, and time on treatment. At a high plane of nutrition and with abrupt introduction of pit water, concentrations of sulfate of 2000 mg/L resulted in reduced dry matter intake without any gross pathological changes. At a low plane of nutrition and with abrupt introduction of pit water, concentrations of sulfate of 1000 mg/L resulted in increased water intake, a response that may pose a problem under hot summer conditions when the availability of a source of non-saline water may be critical. At sulfate concentrations up to 4000 mg/L, cattle adapted by drinking less water, although previous experience of pit water did have some bearing on this response. With gradual introduction there were no effects up to 2000 mg sulfate/L, independent of plane of nutrition.

In Expt 1, there were no accumulations of salts within the muscle that would indicate limitations in the animals' ability to excrete the salts. This is consistent with our earlier work (Robertson *et al*. 1996), which indicated that, with the exception of small amounts of Na, there was no retention of minerals in steers consuming pit water.

The duration and magnitude of this study made introduction of some potential confounding facts unavoidable. For example, 2 batches of coal mine pit water were used in the experiments. Whilst treatment waters were diluted with town water so that the concentration of sulfate was accurately defined, the 40% higher total dissolved solids in the second batch of pit water is

a potential confounding factor. The main effect of nutrition was highly significant for both ADWG and PCV. Given that there were no treatment main effects on ADWG, PCV, or faecal dry matter content at 2000 mg/L or 3000 mg/L, a difference in total concentration of solids of even 40% over the 2000 mg sulfate/L may be of minor significance to the conclusions of Expts 1–4. In terms of the transient physiological stress induced by abrupt introduction to pit water, the difference between batches could have contributed to the significant effects measured, and is worthy of further study.

The time of year at which the experiments were performed is a second potential confounding effect. Whilst Expts 1, 3, 4, and 1*a* were performed during the cooler months, Expt 2 was performed in January, which is generally hot. This may account for the slight difference in water intake within the control groups of Expts 1 and 2.

Standards for the maximum recommended concentrations of minerals in drinking water of livestock have been arbitrarily set. The information on which these are based has often been casual field observations where a cause was assigned to a mineral content of the drinking water without verification. Recent Australian water quality documentation (ANZECC 1992) has highlighted the need for the recommendations to be reassessed after rigorous scientific experimentation. This and a previous publication (Robertson *et al*. 1996) are an attempt to rectify that situation, at least for sulfate.

This study therefore provides evidence to suggest that the recommendation of 1000 mg sulfate/L as the maximum concentration in livestock drinking water may be conservative in circumstances where high mineral waters can be introduced gradually to cattle. Any revised recommendation, however, should take into consideration increased water consumption due to lactation, high ambient temperatures, or the expected duration of exposure to high mineral waters. O'Kelly and Reich (1981) found that when the ambient temperature increased from 24 to 32°C, water intake of steers rose by approximately 30%. If this were applied to the current experiment, the increase in sulfate ingestion would be equivalent to a concentration of 2600 mg/L in the same volume, which suggests that an increase of the recommended level to 2000 mg/L may be inappropriate for temperatures outside the thermoneutral range. A limit of 1500 mg/L may be more appropriately recommended in circumstances where the pit water can be introduced gradually and for not more than 3 months duration, when the temperatures are high. Whilst this change in the recommendations may seem minor in terms of mineral content, the difference may become

significant to a cattle producer paying for water to be transported during drought.

Further work is required to address several issues raised by this study. Firstly, what is the nature of the historical effects that appeared as covariates in Expt 1a, and how long do they last in practice? Secondly, what are the effects of high sulfate pit water on steers over extended periods of time, particularly with respect to animal health? Thirdly, what interactions occur between sulfate and other ions in pit water in terms of detrimental effects to animal health and production as well as human food safety.

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Appendix 1. Haematological data of steers drinking various dilutions of coal mine pit water measured at Weeks 1, 3, 9, and 13 of final concentration

	Normal ranges[A]	Control	Sulfate 500 mg/L	Sulfate 1000 mg/L	Sulfate 2000 mg/L	s.e.m.	Significance
Haemoglobin (g/100 mL)	9·7–14·2	12·8	13·0	13·0	11·1	0·45	<0·001
Packed cell volume (%)	30–43	39·2	40·5	40·5	35·3	1·41	<0·05
$10^{-12} \times$Erythrocytes (per L)	6·8–10	8·6	8·4	8·4	7·3	0·40	n.s.
Mean cell volume (fL)	44–55	45·5	48·6	48·6	48·6	1·62	n.s.
Mean cell haemoglobin	14–20	14·8	15·6	15·6	15·2	1·03	n.s.
MCHC[B] (g/100 mL)	30–36	32·7	32·2	32·2	31·3	0·40	n.s.
Leucocytes ($\times 10^9$/L)	6·6–18·0	12·4	12·6	12·6	13·0	0·89	n.s.

n.s., Not statistically significant at $P = 0·05$.
[A]From the Veterinary Laboratory Users Manual, 5th Edn, Govt Press, Brisbane 1992.
[B]Mean corpuscular haemoglobin concentration.

Appendix 2. Post-mortem blood biochemistry and haematology of 12 randomly selected steers that drank various dilutions of coal mine pit water for the last 13 weeks of the experiment

CPK, creatine phosphokinase; GLDH, glutamate dehydrogenase; AG, ratio albumin/globulin; AST, asparate aminotransferase; MCHC, mean corpuscular haemoglobin concentration; Gamma GT, gamma glutamyltransferase

	Normal ranges[A]	Control	Sulfate 500 mg/L	Sulfate 1000 mg/L	Sulfate 2000 mg/L	s.e.m.	Significance
Biochemistry							
Calcium (mmol/L)	2·1–2·8	2·37	2·41	2·26	2·27	0·08	n.s.
Total protein (g/L)	60–85	71·47	71·87	70·33	71·07	1·85	n.s.
Globulin (μmol/L)	30–45	37·33	36·47	34·20	38·73	1·82	n.s.
Bilirubin (μmol/L)	1–10	2·33	2·00	2·00	2·67	0·64	n.s.
Urea (mmol/L)	2·0–85	9·17	8·60	8·23	8·10	0·79	n.s.
CPK (IU/L)	10–200	248	1896	243	807	526·1	n.s.
GLDH (IU/L)	0–20	13·00	10·70	15·70	11·70	2·22	n.s.
Magnesium (mmol/L)	0·65–1·30	0·83	0·83	0·83	0·86	0·03	n.s.
Albumin (g/L)	30–45	34·13	35·40	36·13	32·33	1·43	n.s.
AG Ratio	0·7–1·15	0·92	0·98	1·06	0·83	0·07	n.s.
Creatinine (μmol/L)	40–200	94·0	101·3	96·0	90·3	4·39	n.s.
Gamma GT (IU/L)	10–35	10·7	13·0	13·3	10·0	2·19	n.s.
AST (IU/L)	30–170	60·0	69·7	55·7	70·3	8·23	n.s.
Haematology							
Haemoglobin (g/100 mL)	9·7–14·2	13·47	14·83	13·57	13·67	0·90	n.s.
Packed cell volume (%)	30–43	41·3	45·3	42·0	44·7	2·94	n.s.
Erythrocytes ($\times 10^{12}$/L)	5·8–10	8·66	9·32	8·48	9·10	0·72	n.s.
Mean cell volume (fL)	44–55	47·33	48·67	50·00	49·33	1·97	n.s.
Mean cell haemoglobin (pg)	14–20	15·53	15·97	16·13	15·13	0·61	n.s.
MCHC (g/L)	30–36	32·5	32·7	32·3	30·6	0·38	n.s.
Leucocytes ($\times 10^9$/L)	6·6–18·0	8·0	12·5	11·7	8·7	2·00	n.s.
Segmented neutrophils (%)	15–45	33·0	25·3	23·7	24·3	6·24	n.s.
Lymphocytes (%)	45–75	61·7	65·0	65·3	69·3	6·52	n.s.
Monocytes (%)	2–7	2·00	4·00	7·00	4·00	1·22	n.s.
Eosinophils (%)	0–20	3·33	5·00	3·67	2·00	1·70	n.s.

n.s., not significant.
[A]From Veterinary Laboratory Users Manual, 5th Edn, Govt Press, Brisbane, 1992.